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Hermaphroditic Freshwater Clams in the Genus *Corbicula* Produce Non-Reductional Spermatozoa With Somatic DNA Content

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Abstract. Hermaphroditic freshwater clams in the genus *Corbicula* produce non-reductional spermatozoa. The DNA content of spermatozoa was almost identical with that of somatic cells in *C. leana* from Mie Prefecture, Japan. Hermaphroditic *C.* aff. *fluminea* from Saga Prefecture and *C. fluminea* from Taiwan also produce non-reductional spermatozoa. On the other hand, spermatozoa of the dioecious *C. sandai* had half the DNA found in somatic cells. Analysis of chromosome numbers suggests that *C. leana* (3n = 54 in somatic cells and 18 in meiotic cells) from Mie Prefecture and *C.* aff. *fluminea* (2n = 36 in gills and 18 bivalents in meiotic cells) from Saga Prefecture are triploids and diploids, respectively. *C. leana, C.* aff. *fluminea,* and *C. fluminea* may lack either first or second meiosis, resulting in non-reductional spermatozoa. We assume that gynogenetic reproduction occurs in both species; maternal chromosomes are also non-reductional, and spermatozoa activate development of the eggs, but do not contribute to the offspring.

Introduction

The freshwater clam *Corbicula leana* has a special mode of reproduction: it is hermaphroditic and broods its larvae in the inner demibranchs (Miyazaki, 1936; Ikematsu and Yamane, 1977). A recent study of the chromosomes of *C. leana* showed that it has 54 somatic chromosomes, which, judging from its karyotype, are triploids (Okamoto and Arimoto, 1986). Okamoto and Arimoto (1986) suggested the possibility that *C. leana* reproduces by gynogenesis, which would account for the odd chromosome number: *i.e.*, gynogenetic eggs are usually meiotically unreduced, and spermatozoa activate the development of eggs, but the paternal genome does not contribute to the offspring. Analysis of allozyme variation in *C. leana* also revealed that the species has much lower genetic variability than *C. japonica* and *C. sandai* (Sakai *et al.,* 1994). In this study, we compare chromosome numbers and DNA content of somatic cells and spermatozoa in the hermaphroditic *Corbicula* species to test the gynogenesis hypothesis proposed by Okamoto and Arimoto (1986).

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Materials and Methods

The *Corbicula* species were identified according to Habe (1977). *C. leana* and *C.* aff. *fluminea* were collected in Japan from an irrigation canal in Meiwa, Mie Prefecture, and from the Tade River, Saga Prefecture, respectively. In this study we use the name *C.* aff. *fluminea* for clams collected from Saga Prefecture because the morphology of the lateral teeth in these specimens was very similar to that of *C. fluminea* (Komaru *et al.,* 1998). *C. fluminea* was also obtained from a cultured population in Shin Wu, Taiwan. For comparison using microfluorometry, specimens of *C. sandai* from Lake Biwa, Shiga Prefecture, were obtained at the market in Ootsu city.

Chromosomes

The clams were treated with 0.002% colchicine for 4–5 h. Gills and gonads were dissected out with scissors,

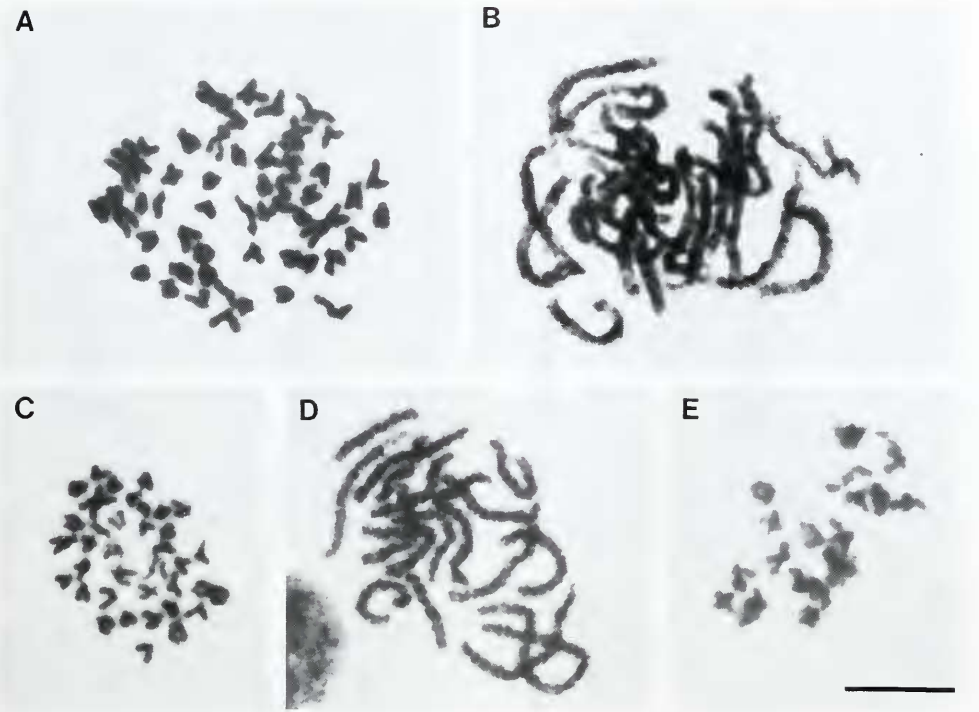

Figure 1. Mitotic (A, C) and meiotic (B, D, E) chromosomes of *Corbicula leana* from Mie Prefecture (A, B) and *C.* aff. *fluminea* from Saga Prefecture (C, D, E). Scale bar = 10 μm.

treated with hypotonic solution (8 m*M* KCl), and fixed with Carnoy's fixative according to the procedure of Okamoto and Arimoto (1986). Cells were isolated from either gill or gonads, placed on slides, and stained with 2% Giemsa in phosphate buffer (pH 7.2). At least 4 clams were examined for each case (*i.e.*, each combination of species and tissue type).

DNA microfluorometry

To compare the relative DNA content of spermatozoa and somatic tissue, cells were isolated on a glass slide by cutting a small piece of gonad or mantle in distilled water with a scalpel and air-drying it before fixing it with 70% ethanol. Spermatozoa and somatic cells from one individual were placed on the same slide. The cells were stained with the DNA-specific dye DAPI, and the relative DNA content (fluorescence intensity) per cell was estimated by microfluorometry as in Komaru *et al.* (1988). Spermatozoa could be easily distinguished from other spermatogenic cells because of their elongate and curved morphology.

Results

Mitotic and meiotic chromosomes

In four specimens of *C. leana*, we counted 54 chromosomes in the mitotic metaphase plates of somatic tissue (Fig. 1A) and about 18 chromosomes in the gonad (Fig. 1B). In *C.* aff. *fluminea*, we observed 36 chromosomes in the mitotic metaphase in 16 cells, but the haploid number of 18 in 8 gonadal cells. Pachytene (Fig. 1D), diakinesis or metaphase I (Fig. 1E) figures were observed in the meiotic cells.

Relative DNA content of spermatozoa and somatic cells

In *C. leana*, *C.* aff. *fluminea*, and *C. fluminea*, the relative content of DNA in the spermatozoa was also identical

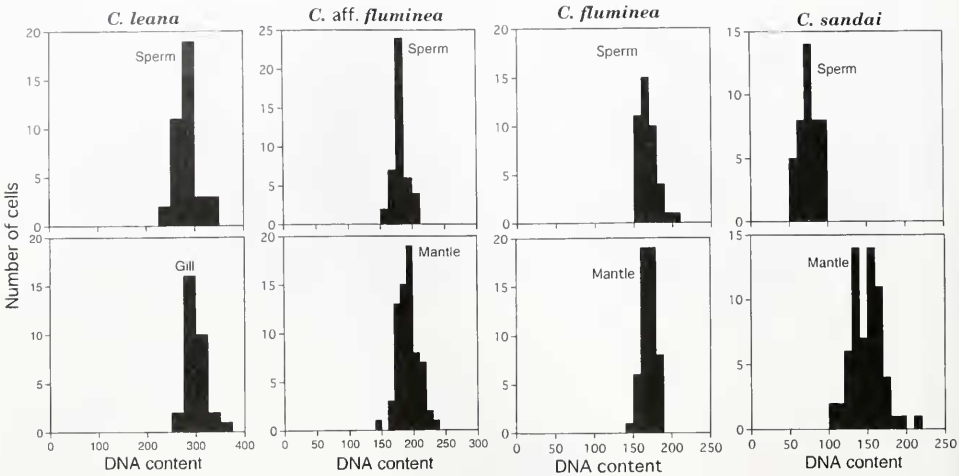

Figure 2. Histograms of fluorescence intensity (relative DNA content) in spermatozoa and somatic cells of *Corbicula leana* from Mie Prefecture, *C.* aff. *fluminea* from Saga, *C. fluminea* from Taiwan, and *C. sandai* from Shiga. Each histogram was derived from one individual. DNA content per cell was measured as fluorescence intensity.

to that in the somatic cells (Fig. 2, and Table I). In *C. sandai*, however, the DNA content of the spermatozoa was half that of the somatic cells.

Discussion

Okamoto and Arimoto (1986) reported chromosome numbers of 3n = 54 for *C. leana*, 2n = 38 for *C. japonica*, and 2n = 36 for *C. sandai*. In the present study, the chromosome number of *C.* aff. *fluminea* was determined to be 2n = 36 in gill tissue and 18 bivalents in gonads, indicating that *C. fluminea* is a diploid species. In contrast, *C. leana* has a somatic chromosome number of 54, but in the gonads, three homologous chromosomes form 18 trivalents. The synapsis of three homologous chromosomes should be incomplete. These results suggest that *C. leana* is a triploid species.

Table I

Relative DNA content of spermatozoa and somatic cells in three species of Corbicula

Species	Sample no.	DNA content (mean ± SD) Somatic cell	Spermatozoa
C. leana	1	251.6 ± 23.6 (n = 94)	251.2 ± 24.8 (n = 53)
	2	259.5 ± 17.4 (n = 66)	278.5 ± 45.2 (n = 16)
	3	295.0 ± 25.4 (n = 119)	328.5 ± 29.0 (n = 26)
	4	315.5 ± 32.6 (n = 28)	317.9 ± 36.6 (n = 23)
C. aff. *fluminea*	1	192.4 ± 17.6 (n = 70)	181.9 ± 12.5 (n = 44)
	2	176.0 ± 10.8 (n = 22)	175.5 ± 18.9 (n = 24)
	3	204.0 ± 18.3 (n = 48)	203.8 ± 10.1 (n = 48)
	4	183.7 ± 13.5 (n = 39)	179.6 ± 13.1 (n = 56)
C. fluminea	1	160.6 ± 12.1 (n = 27)	161.0 ± 13.3 (n = 26)
	2	172.4 ± 12.9 (n = 53)	191.2 ± 13.7 (n = 26)
	3	151.1 ± 7.9 (n = 54)	168.5 ± 10.5 (n = 34)
	4	161.3 ± 11.2 (n = 41)	166.0 ± 9.7 (n = 40)
C. sandai	1	202.8 ± 12.8 (n = 36)	109.3 ± 6.4 (n = 38)
	2	164.1 ± 14.5 (n = 53)	90.3 ± 8.2 (n = 38)
	3	148.3 ± 20.0 (n = 63)	75.7 ± 12.2 (n = 38)
	4	180.8 ± 11.3 (n = 34)	99.3 ± 15.3 (n = 16)

Each mean value and standard deviation (SD) was derived from one individual. The numbers in parentheses (n) are the number of cells measured.

The microfluorometric measurements of DNA revealed that the hermaphroditic species *C. leana* and *C. fluminea* produced non-reductional spermatozoa; *i.e.*, sperm and somatic cells had the same DNA content. On the other hand, the dioecious *C. sandai* produced reductional spermatozoa; the DNA content of sperm was half that of somatic cells. These results suggest that the cytokinesis of first or second meiosis in the spermatocytes of both *C. leana* and *C. fluminea* is abortive, and only one equal division occurs. Consequently, meiosis during spermatogenesis may be non-reductional, resulting in spermatozoa with somatic ploidy levels.

It is possible that triploid *C. leana* and diploid *C. fluminea* reproduce by gynogenesis, as O'Foighil and Thiriot-Quiévreux (1991) observed in the bivalve *Lasaea*. In gynogenetic development, spermatozoa stimulate the development of eggs but do not contribute paternal genome to the offspring. In this case the somatic chromosome number may be restored to the eggs by premeiotic endomitosis

or abortive meiosis (Cuellar, 1987). How did the triploid *C. leana* evolve from a diploid ancestral species? We assume that the diploid gynogenetic *C. fluminea* arose first. Later, the triploid *C. leana* may have evolved from the diploid gynogen by fusion of diploid and haploid gametes. The processes of meiosis and fertilization of eggs should be observed in *C. leana* and *C. fluminea* to understand the reproductive processes in these species with non-reductional spermatozoa.

Species of *Corbicula* show polymorphic shell color and morphology (Kuroda, 1938; Morton, 1986). The taxonomy of the Corbiculidae is in disarray as a result of intraspecific variation in shell morphology and insufficient biological information. *Corbicula* taxonomy cannot be clarified on the basis of shell morphology alone; as Morton (1986) suggested, ecological, genetic, and physiological studies will also be necessary. If the genus *Corbicula* includes polyploid species that reproduce by gynogenesis, as our results indicate, it will be difficult to apply the biological species concept to these clams (Mayr and Ashlock, 1991). The definition of species in *Corbicula* should be addressed after sufficient data on reproductive biology have been collected.

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